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"ANALYSIS OF VITAMIN D & VITAMIN B12 STATUS DURING 1st TRIMESTER OF PREGNANCY IN A TERTIARY CARE CENTRE''

DISSERTATION SUBMITTED FOR

THE AWARD OF M.Sc DEGREE IN MASTER OF SCIENCE

IN THE SPECIALTY OF MEDICAL BIOCHEMISTRY

By

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M.Sc MEDICAL BIOCHEMISTRY

(2021-2024)

UNDER THE GUIDANCE OF

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DECLARATION BY THE CANDIDATE

I hereby declare that thesis**"ANALYSIS OF VITAMIN D & VITAMIN B12 STATUS DURING 1st TRIMESTER OF PREGNANCY IN A TERTIARY CARE CENTRE"**is a bonafide genuine research work carried by me, under the esteemed guidance of my guide

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INTRODUCTION

Our proposed research aims to investigate the connection between vitamin D and vitamin B12 status in the first trimester of pregnancy at a tertiary care center.Deficiency of vitamin D & vitamin B12 are widespread, posing potential risks during pregnancy[1]**.** This study aimed to investigate the prevalence and potential consequences of such deficiencies in pregnant women, particularly in the first trimester. A decrease in bone mineral content in the fetal skeleton can occur if there is a lack of vitamin D during pregnancy and special attention in the early stages[2]**.**Vitamin D is essential for achieving optimal pregnancy outcomes and supporting tissue growth and function in the developing fetus through proper metabolic activities. Vitamin D deficiency during pregnancy has been identified as a high-risk group that includes vegetarians and women with limited sun exposure[3]**.**Severe maternal vitamin D deficiency during pregnancy can be identified by serum 25-hydroxyvitamin D concentrations less than 5ng/ml, as defined by the term severe maternal vitamin D deficiency[4]**.**The risk of complications can be minimized by aiming for vitamin B12 concentrations of 221pmol/l. The pregnancy outcome for both mother and offspring can be affected by maternal vitamin B12 deficiency, so it's important to maintain a healthy vitamin B12 status during pregnancy[5]**.** Vitamin B12 and folate act as methyl donors in one-carbon metabolism which affects cell growth and differentiation by influencing DNA synthesis and epigenetic regulation. Therefore, they are important regulators of fetal growth.Preeclampsia, intra-uterine growth retardation, and preterm labor are all possibilities for women who want to become pregnant due to a vitamin B12 deficiency. Low vitamin D & vitamin B12 levels are associated with increased risk of diseases like T2D, CVD and low birth weight.The prevalence of hypertension in Pregnancy period women is estimated to be 7.7%[6]**.**Several diseases, including oxidative stress, such as kidney diseases, diabetes, cardiomyopathies, and Alzheimer's disease, are correlated with vitamin D deficiency. Vitamin D & vitamin B12

deficiency in pregnant women can negatively impact both maternal and fetal health, leading to issues like bone problems, preeclampsia, and low birth weight. Vitamin D & vitamin B12 plays a crucial role in various physiological processes during pregnancy[7]**.** Intrauterine growth restriction, spontaneous abortion, gestational diabetes, and preterm delivery are just some of the outcomes that have been linked to deficiencies or insufficiencies[8]**.** The process of selecting participants from a community or recruiting them from a specific setting like a workplace or healthcare facility could involve random selection. This information can be utilized to identify potential risk factors for vitamin deficiencies[9]**.** The manufacturer's protocol for COBAS e411 requires the use of established laboratory methods like electrochemiluminescence assay (ECLIA) kits to analyze vitamin D and vitamin B12.This study was based on vitamin D and vitamin B12 kit principle.The principle can aid in determining if you have deficiencies, excessive vitamin D, or how your body reacts to vitamin B12 therapy[10]**.**The results of such a study can be beneficial in providing valuable insights into the prevalence of vitamin D and B12 deficiency in a population and identifying potential risk factors[11]**.**

REVIEW OF LITERATURE

ROLE OF VITAMIN D IN PREGNANT WOMEN IN FIRST TRIMESTER

It is common to have a deficiency of 25-hydroxyvitamin D (vitamin D) and vitamin B12 worldwide. Studies have observed connections between low vitamin D and an increased risk of several chronic diseases[12]**.** Vitamin D plays a vital role in bone health, immune function, and cell growth. It helps your body absorb calcium, which is crucial for strong bones. It also seems to influence various other systems in the body.

Vitamin D supplementation has been hypothesized to be effective in preventing both type 2 diabetes (T2D) and cardiovascular disease (CVD).Type 2 Diabetes: Studies have observed a link between low vitamin D and a higher risk of T2D[13]**.**Vitamin D might play a role in insulin secretion and sensitivity. However, results from large trials on supplementation for prevention are mixed. Some show benefit, particularly for those without obesity, while others don't. Cardiovascular Disease: Similar to T2D, research suggests a connection between low vitamin D and increased risk of CVD. Vitamin D might influence factors like blood pressure and inflammation, both linked to CVD[14]**.** There's some evidence that supplementation may help, but more research is needed. Many countries are facing a major public health problem due to the increasing prevalence of vitamin D deficiency, Even in sun-rich areas, pregnant women exhibit a high prevalence of vitamin D deficiency [15]**.**The primary source of vitamin D in our body is sunlight, and when our skin is exposed to ultraviolet B (UVB) rays from the sun, it triggers the production of vitamin D3 (cholecalciferol), the most usable form of vitamin D by the body**(3).** Vitamin D insufficiency and deficiency are on the rise in pregnant women around the world, and there are a few reasons why:

- Less sun exposure: Modern life often keeps us indoors for work, leisure, and sun protection. Sunlight triggers vitamin D production in the skin, so limited sun exposure reduces our natural source.
- Dietary factors: While some foods are rich in vitamin D, they may not be a regular part of everyone's diet. Additionally, some people follow restricted diets that might be lower in vitamin D.
- Increased risk groups: Certain ethnicities, those with darker skin pigmentation, and pregnant women with pre-existing health conditions may be more susceptible to deficiency.

This rise in vitamin D deficiency during pregnancy is concerning because it's linked to potential health problems for both mother and baby[16]. Clinical investigations showed that vitamin D was essential to the health. Pregnancy can cause a decrease in bone mineral content in the fetal skeleton due to a low level of vitamin D and specific attention during the early stage of pregnancy. Cord blood typically has lower levels of calcitriol compared to maternal serum.Low vitamin D in pregnancy can impact fetal bones: If a pregnant woman has low vitamin D levels, her baby's bones may not develop as much bone mineral content in the early stages. This can affect bone health later in life.

Calcitriol levels are lower in cord blood: Calcitriol is the active form of vitamin D. The research suggests that babies receive less calcitriol through the umbilical cord compared to the mother's blood levels. Doctors often recommend vitamin D supplements for pregnant women to ensure their babies get what they need for proper bone development.

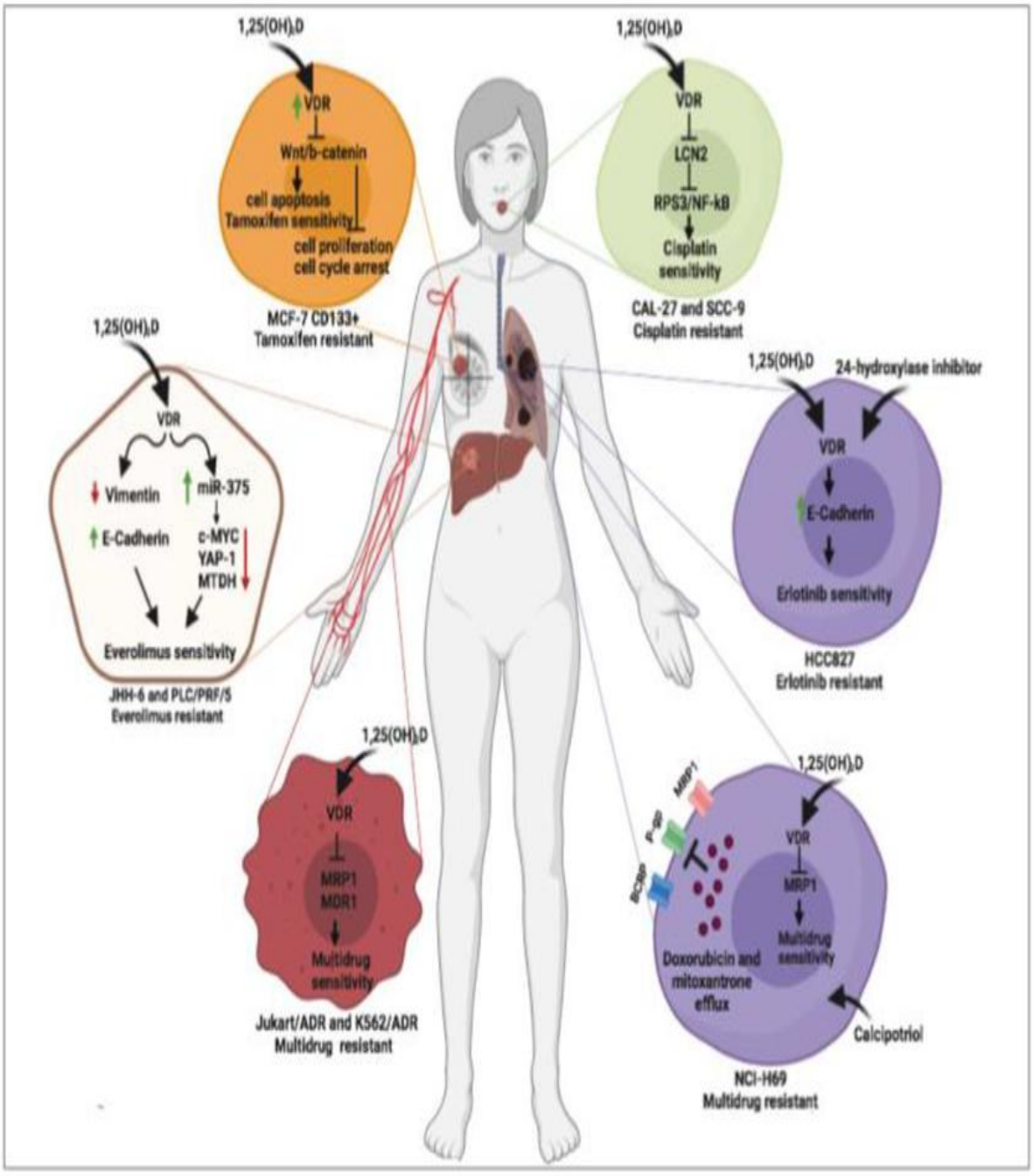

Figure 1. Vitamin D-induced molecular mechanisms involved in reversing drug-resistance in several effect: oral, breast, liver and lung affecting.

A significant negative impact on the health of pregnant women occurs when vitamin D levels are insufficient or deficient during pregnancy. Vitamin D helps regulate calcium, essential for strong bones. Deficiency can be linked to an increased risk of pregnancy complications like preclampsia and gestational diabetes. Vitamin D is crucial for fetal bone development Serum 25-hydroxyvitamin D (25-OH vitamin D) concentrations less than 5 ng/ml is considered a severe vitamin D deficiency and has been linked to several skeletal problems in fetus. This refers to an imbalance in the body's processes of building and breaking down bone. Vitamin D is essential for calcium absorption, which is crucial for bone health. Without enough vitamin D, bones can become weak and brittle[17].Therefore, checking vitamin D status in the first trimester offers a window of opportunity for early detection and prevention of potential problems associated with deficiency. The optimal level of vitamin D for pregnant women is still being debated, with recommendations ranging from 20 ng/mL to 30 ng/mL. Therefore, checking vitamin D status in the first trimester offers a window of opportunity for early detection and prevention of potential problems associated with deficiency. Studies show a significant portion of pregnant women have vitamin D deficiency or insufficiency in the first trimester .Low vitamin D levels during pregnancy have been linked to increased risks of gestational diabetes mellitus (GDM), preeclampsiaand very bad impact on fetus.

A pregnancy complication with high blood pressure and potential organ damage. Gestational diabetes: High blood sugar levels that develop during pregnancy. Preterm delivery: Delivering a baby before 37 weeks of gestation. Spontaneous abortion: Miscarriage. Growth restriction: When the baby's growth falls below expected levels[18].Severe maternal vitamin D deficiency, where serum 25-hydroxyvitamin D (25-OH vitamin D) concentrations are lower than 5 ng/ml, has been linked to disordered skeletal homeostasis during pregnancy., Has been linked to disordered bone health, congenital rickets, and fractures in young infants.Vitamin D is essential for helping the

body absorb calcium, which is crucial for building strong bones. If a pregnant woman is severely deficient in vitamin D, her baby may not get enough calcium to develop healthy bones[19].

The liver is the primary source of hydroxylation for 25(OH)vitamin D. To measure Vitamin D status, the most commonly used metabolite is 25(OH)vitamin D because it is very stable. The second hydroxylation to the active form 1,25(OH)D occurs mostly in the kidneys in a process tightly regulated by calcium, phosphorus and parathyroid hormone levels.Sun exposure or dietary intake: Your body gets vitamin D from sun exposure on your skin or through vitamin D-rich foods. Liver conversion: The liver converts vitamin D into 25(OH)vitamin D , the inactive form. Storage and measurement:This stable metabolite circulates in the bloodstream and gets stored in fatty tissues. Due to its stability, measuring 25(OH)vitamin D levels is the most reliable way to assess your overall vitamin D status.The kidneys further convert 25(OH)vitamin D into the active form, 1,25-dihydroxyvitamin D, which your body uses for various functions. Several factors can affect your vitamin D levels, such as sun exposure, diet, and certain medical conditions[20].1,25(OH)D, also known as calcitriol, plays a crucial role in the body through its interaction with the VDR.1,25(OH)D Binding: After undergoing two hydroxylation, vitamin D becomes 1,25(OH)D, the most active form. This form binds specifically to the Vitamin D Receptor (VDR).

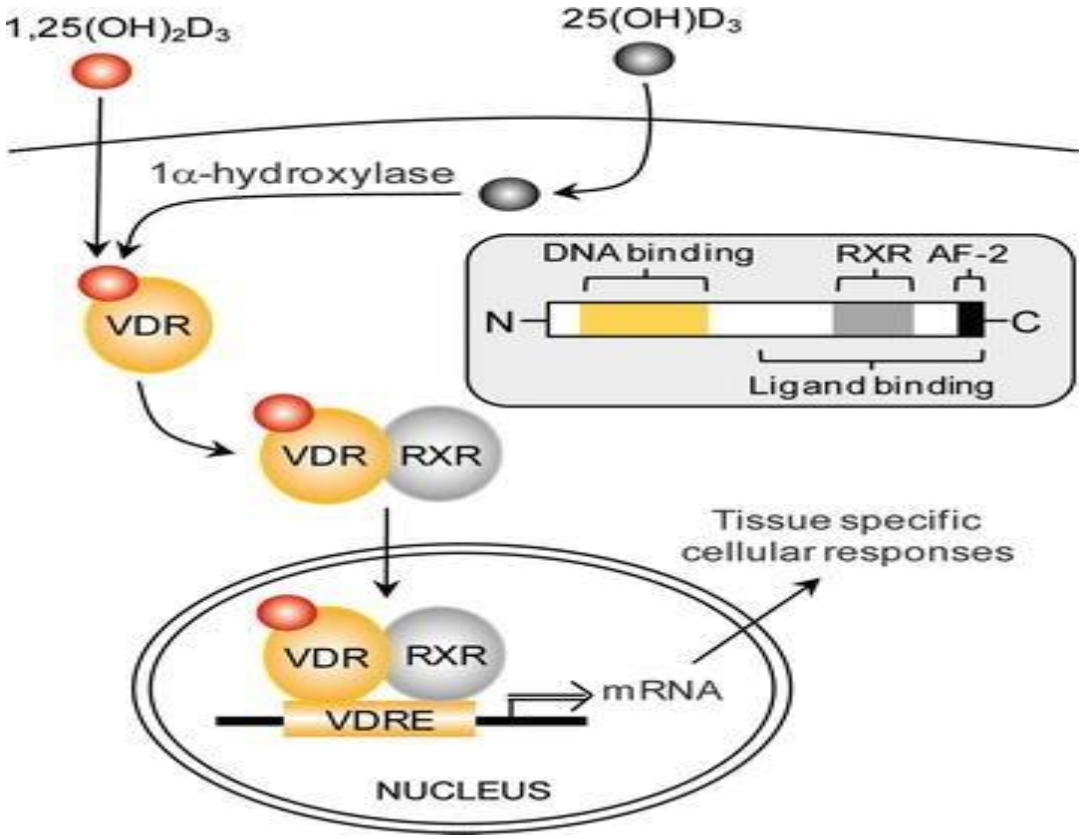

Figure 2. ***Vitamin D receptor (VDR) action at target cells. Intracellular [1,25(OH)2D3] binds to the VDR; thus, causing its dimerization with the retinoid X receptor (RXR). The ligand-bound VDR-RXR complex binds to structurally distinct vitamin D response elements (VDREs) in multiple, widely spaced vitamin D-responsive regions, which leads to positive or negative transcriptional regulation of gene expression (von Essen et al.,2012).***

VDR as a Transcription Factor: VDR is a protein that resides inside the cell nucleus. When 1,25(OH)D binds to VDR, it acts as a transcription factor. Transcription Factor Function: Transcription factors regulate gene expression. Upon binding 1,25(OH)D, VDR can turn on or off specific genes by interacting with their DNA.

Wide Range of Effects: The genes VDR regulates are involved in various processes throughout the body, including:

- Bone Metabolism: Maintaining strong and healthy bones is a classic function of vitamin D. VDR helps regulate genes involved in calcium and phosphate absorption, crucial for bone mineralization.

- Cellular Growth & Differentiation: VDR plays a role in controlling how cells grow, mature, and specialize into different types.
- Glucose Metabolism: VDR can influence how the body processes sugar (glucose) for energy.
- Immune Function**:** A healthy immune system relies partly on VDR activity to function properly[21]**.**

The presence of both 1α-hydroxylase (the enzyme that activates vitamin D) and its receptor in peripheral tissues like the placenta points to a wider range of functions for vitamin D beyond just bone health.Local Vitamin D Activation**:** Classically, vitamin D is activated in the kidneys. This suggests that vitamin D can have localized effects in these tissues independent of the kidney.

Diverse Functions: The presence of the vitamin D receptor in these tissues indicates the ability of 1,25(OH)2D3 to directly influence cellular processes. This implies vitamin D has roles beyond just regulating calcium homeostasis and bone metabolism, potentially impacting functions like cell growth, differentiation, and immune regulation in various organs.The placenta, is a great case in point.Local vitamin D action might be crucial for fetal development and immune function[22]**.**

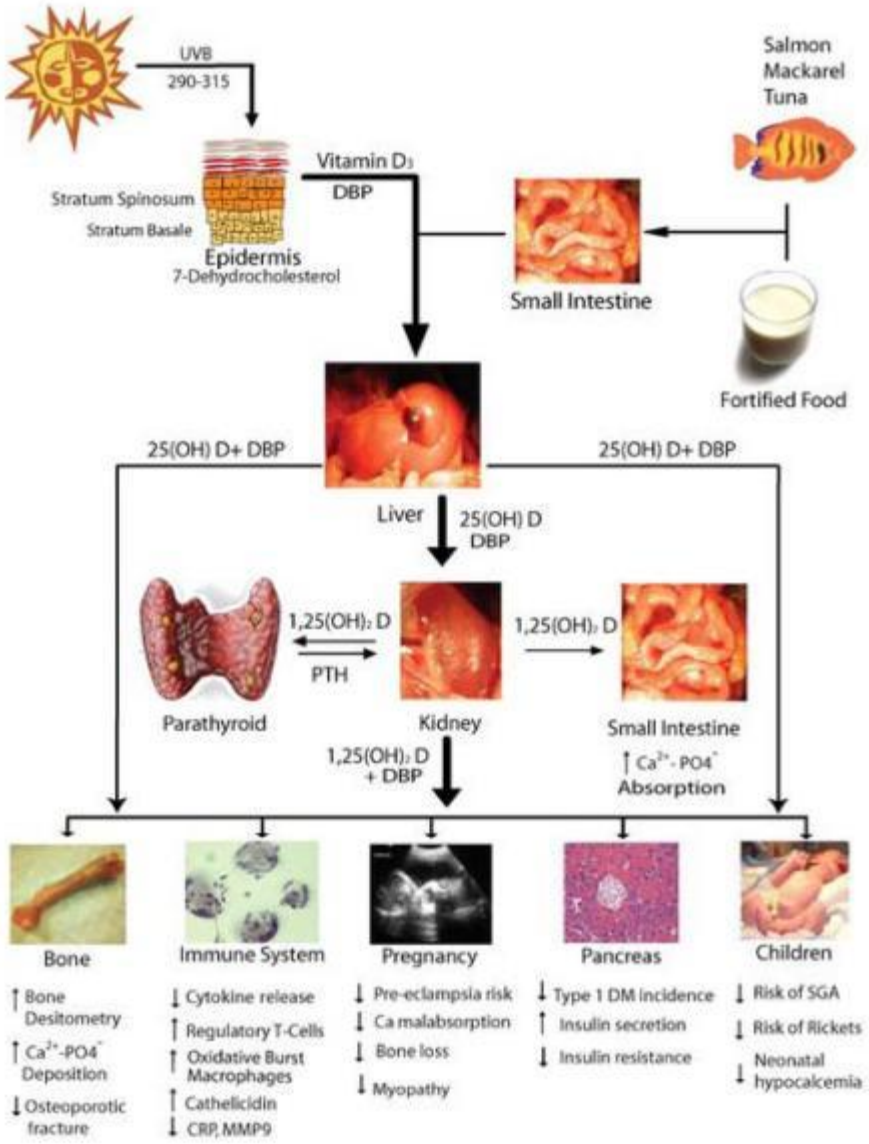

Figure 3: Production, metabolism and functions of vitamin D (Alshishtawy et al.,2012)

Vitamin D deficiency is a major concern in pregnant women, especially in North India. Studies have shown a very high prevalence of vitamin D deficiency among pregnant women in this region, ranging from 93% to 97% (12). Even though it is reported that vitamin D deficiency during pregnancy has a high prevalence and risk, Optimizing vitamin D status during pregnancy has been the focus of only a few RCTs[23]**.** North India is one of the most high-risk

populations.High Prevalence, High Risk:Vitamin D deficiency seems common among pregnant women in many regions, including North India. This deficiency is linked to potential complications during pregnancy. Limited RCTs: Despite the established risks, there are only a few Randomized Controlled Trials (RCTs) specifically focused on optimizing vitamin D levels in pregnant women through supplementation. Research specifically targeted at high-risk populations like pregnant women in North India is crucial to understand how supplementation can best address their needs[24].

ROLE OF VITAMIN B12 IN PRAGNENT WOMEN IN FIRST TRIMESTER

Vitamin B12 is an important nutrient for both mother and baby during pregnancy. It plays a crucial role in fetal neural tube development, red blood cell formation, and DNA synthesis.

- Importance: Vitamin B12 is essential for healthy cell growth and development of the nervous system. Red blood cells, which transport oxygen throughout the body, are also formed by it.
- Potential risks of deficiencies: Studies have shown that low vitamin B12 levels during pregnancy may be associated with an increased risk of neural tube defects, such as spine bifida, in the developing baby. It can also increase the risk of preterm birth and low birth weight.
- Changes in vitamin B12 levels: There is a natural decrease in serum vitamin B12 levels throughout pregnancy due to increased blood volume and hormonal changes. However, this doesn't necessarily indicate deficiency.
- Checking vitamin B12 levels: There is no standard reference range for vitamin B12 specifically for pregnant women. Doctors may recommend a blood test to check vitamin B12 levels, especially if you have a risk of deficiency, such as being vegetarian or vegan, or having a history of digestive disorders[25].

Dietary Restrictions**:** People who don't consume enough animal products, like vegetarians and vegans, are at higher risk if they don't have alternative sources of B12, such as fortified foods or supplements. Age:As we get older, Women bodies may not absorb B12 from food as efficiently. This is especially true for women over 50. Medical Conditions: Certain medical conditions can affect how well your body absorbs B12. These include pernicious anemia, a condition where the stomach doesn't produce enough intrinsic factor (a protein needed for B12 absorption), and Crohn's disease, which can damage the part of the intestine responsible for B12 absorption[26]**.** This pathway is essential for producing building blocks needed for DNA synthesis, cell division, and growth. B12 and folate act as methyl donors, providing the crucial methyl groups for these processes.Proper DNA formation is vital for fetal development. Deficiencies in B12 and folate can disrupt DNA replication, leading to abnormal cell growth and potentially birth defects. This refers to changes in gene expression that don't alter the DNA sequence itself. B12 and folate influence these epigenetic modifications, ensuring proper development of organs and tissues in the growing fetus[27]**.**

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Increases before delivery: It's good to know that the concentration starts to rise again before delivery, returning to normal levels after birth. Stable throughout pregnancy: It's interesting that

the concentration of the active form, holotranscobalamin, remains stable. This suggests that even though total B12 concentration dips, the body might be prioritizing converting it to the active usable form [28].221 pmol/L (around 300 pg/mL) - This level has been proposed as a good starting point for pregnancy to minimize complication risks. Above 295 pmol/L (around 400 pg/mL) - This concentration may be even better for reducing pregnancy complications[29].

Vitamin B12 plays a vital role in fetal development and red blood cell production. Deficiency can lead to complications for both mother and fetus. Vitamin B12 can be found in meat, poultry, fish, eggs, and dairy products Vegetarians & vegans may need to consider supplementation[30].Vitamin B12 levels naturally dip during pregnancy due to physiological changes. Using the same cut-off levels for diagnosing deficiency as in non-pregnant individuals would misclassify many pregnant women. There's a lack of research on ideal B12 levels specifically for optimal pregnancy outcomes. Establishing pregnancy-specific thresholds would give doctors a more accurate picture. Relying on non-pregnant cut-offs could lead to over-diagnosing B12 deficiency in pregnant women[31]. This might cause unnecessary treatment and anxiety.

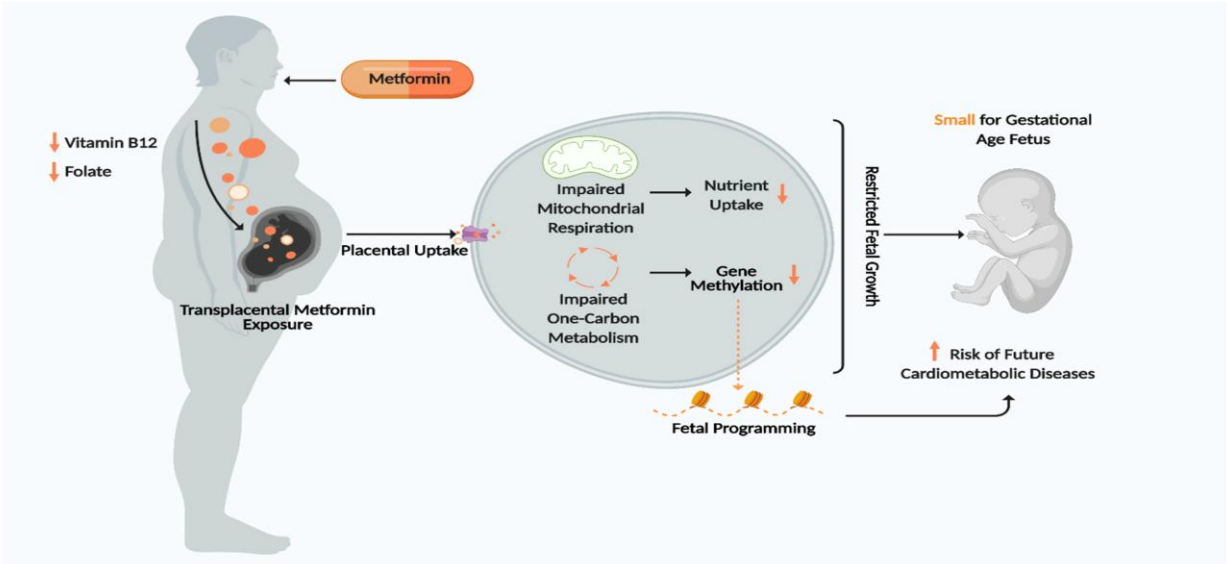

Figure 4. Summary of Proposed Mechanism for metformin effects on placenta and fetus. Figure created using Biorender.com (Owen et al.,2021).

The development of the developing fetus can be seriously impacted by micronutrient deficiencies during pregnancy. Symptoms may not be present in every patient with a vitamin B12 deficiency, even though it has been observed worldwide recently. Only very severe deficiency results in hematological signs of vitamin B12 deficiency[32].The data shows that vitamin B12 deficiency in developing countries does not correlate with megaloblastic anemia globally.The pregnancy outcome for both mother and offspring can be affected by maternal vitamin B12 deficiency, which is why it is crucial to maintain vitamin B12 status during pregnancy. For women who want to get pregnant, a vitamin B12 deficiency means an increased risk of developing preeclampsia, intra-uterine growth retardation, preterm labor[33].

Neural tube defects are strongly associated with low vitamin B12 status in mothers.The presence of a deficient or inadequate vitamin B12 status during pregnancy may increase the risk of birth defects, but there is no evidence of a causal relationship[34].A major concern is an increased risk of neural tube defects [NHS]. The development of the brain and spinal cord is impacted by these serious birth defects. Such as spine bifida. B12 plays a crucial role in this development, and deficiency during critical early stages can lead to malformations. B12 is crucial for fetal development, particularly for the nervous system. If a mother is deficient, the baby may be born with low B12 stores, which can lead to developmental delays and neurological problems later on[35].

Severe vitamin B12 deficiency in infants can be quite serious. It can impact their development and overall health in several ways. Here's a breakdown of what you mentioned. B12 is crucial for proper nervous system function. Deficiency can lead to irritability, apathy (lack of interest), developmental delays, and even seizures in severe cases. This refers to poor weight gain and growth in infants. B12 deficiency can affect their appetite (anorexia) and hinder their ability to absorb nutrients properly.

Vitamin B12 is essential for red blood cell production. Megaloblastic anemia, which occurs when red blood cells are larger than normal and less functional, can result in fatigue and paleness due to a lack of nutrition [36].Studies have found associations between low B12 in pregnant women and including impaired growth of fetus, poor motor skills, cognitive difficulties, Vitamin B12 is crucial for fetal brain development and nervous system function. Deficiency during pregnancy may hinder these processes, potentially leading to the observed issues[37].

The increase in metabolic demands imposed by physiological activities makes women more susceptible to a deficiency of vitamin B12 during pregnancy, Among other factors, the placenta, fetus, and maternal tissue grow[38].People who follow vegetarian or vegan diets, for ethical or cultural reasons, are at an increased risk. This is because vitamin B12 is naturally found in animal products In low-income countries, people may not be able to afford or have access to a varied diet that includes animal source foods like meat, dairy and eggs, Vitamin B12 supplements are available and can help ensure adequate intake for people at risk of deficiency[39].

Bacterial, virus, or parasites can cause gastrointestinal infections by damaging the lining of the small intestine where vitamin B12 are absorbed[40]. This is a common bacterial infection of the stomach that can reduce stomach acid production and interfere with vitamin B12 absorption. Celiac diseases are an autoimmune disorder that triggers an immune response when gluten (a protein found in wheat, barley, and rye) is consumed[41].This damages the small intestine and hinders nutrient absorption, including vitamin B12. This is an inflammatory bowel disease (IBD) that can cause inflammation throughout the digestive tract, including the small intestine, which can impair nutrient absorption pregnant Bangladeshi women are at risk of vitamin B12 deficiency and marginal deficiency, but there is limited data available[42].

The prevalence of vitamin B12 deficiency increases significantly as pregnancy progresses, as revealed in this study[43].

Women with a gestational age of <unk>27 weeks faced a risk of marginal vitamin B12 deficiency that was almost twice that of women with a gestational age of <unk>27 weeks, There was no statistical significance to the difference[44]. During late pregnancy, Dutch women were studied and it was found that dairy was the best source of vitamin B12 intake. Plasma levels of total vitamin B12 were influenced by meat, fish, but not eggs through a dose-response relationship [45].

Source of Vitamin B12: Vitamin B12 in animal products is bound to protein. Meat, fish, and dairy contain different types of protein which might affect how well the B12 is absorbed by the body. Eggs might have a protein structure that makes releasing B12 less efficient.

Amount of Vitamin B12:It's possible that eggs naturally contain lower amounts of B12 compared to dairy, meat, and fish. This could explain why higher B12 intake from those sources had a stronger effect[46].

The research by Yajnik and colleagues is significant because it suggests a link between maternal vitamin B12 deficiency in early pregnancy (first trimester) and increased insulin resistance and fat accumulation (adiposity) in the offspring. This is noteworthy for a couple of reasons[47]. Vitamin B12 is essential for fetal development, especially during the first trimester when the neural tube is forming. A deficiency in vitamin B12 can lead to a rise in the chance of neural tube defects, which are severe birth defects that affect the brain and spinal cord[48].

Vitamin B12 deficiency is a concern:The study suggests low B12 levels in pregnant women are associated with negative outcomes.

The risks identified:The analysis linked B12 deficiency to an increased risk of both preterm birth (delivery before 37 weeks) and low birth weight (LBW) babies[49].

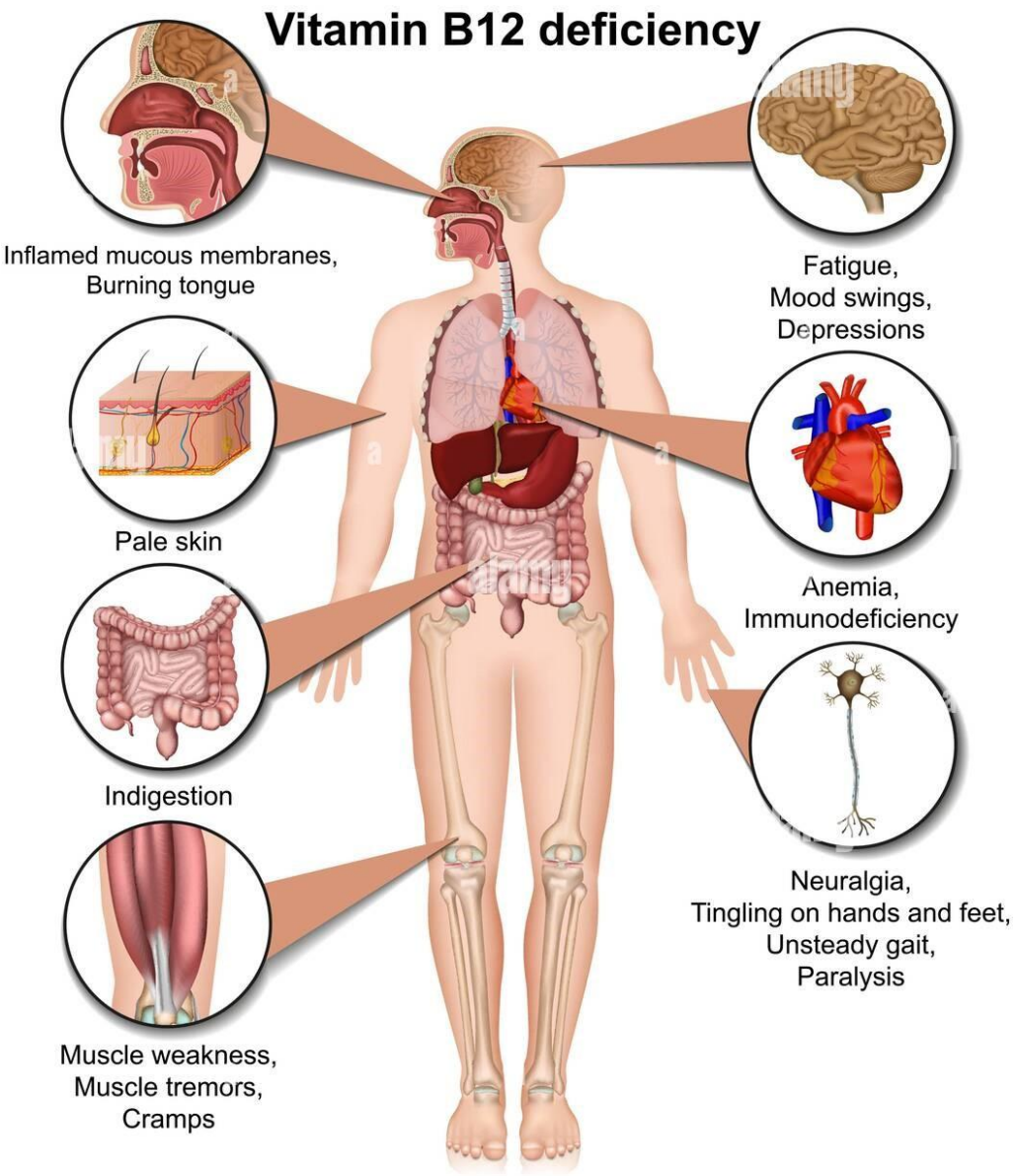

Figure 5: Vitamin B12 deficiency and key functions in human physiology

Folic acid and vitamin B12 are essential for the formation of the neural tube, which ultimately becomes the brain and spinal cord of the baby.Deficiency in either B12 or folate during pregnancy increases the risk of neural tube defects such as spine bifida. Vitamin B12 is passed on to the baby through breast milk. If a mother is deficient in B12, the concentration in her breast milk will also be low, potentially affecting the infant's B12 stores[50].

Low vitamin B12 levels have been suggested as a risk factor for CVD and diabetes.There's some suggestion that in people with type 2 diabetes, very low B12 levels might be linked to a higher risk of death from CVD. Interestingly, extremely high B12 levels might also be associated with increased risk[51].Vitamin D is crucial for a healthy pregnancy. Calcium absorption is essential for strong bones and teeth, and it helps the developing baby do it.Vitamin D deficiency during pregnancy can lead to a number of health problems for both the mother and fetus[52]. Low vitamin D levels are associated with an increased risk of various health problems like kidney disease, diabetes, heart muscle problems (cardiomyopathy), and Alzheimer's disease. Oxidative stress occur when there's an imbalance between free radicals reactive oxygen species, (ROS) and antioxidants in the body. This imbalance can damage cells and contribute to disease development. Vitamin D helps regulate cellular pathways involved in antioxidant defenses[53]. When vitamin D is deficient, the body's ability to combat free radicals and oxidative stress gets compromised, potentially leading to increased cell damage[54].Hypertension, a leading cause of maternal mortality, morbidity, fetal growth restriction, and preterm birth, can be caused by insufficient vitamins during pregnancy.Hypertension (high blood pressure): This is a major concern, as it can increase the risk of preeclampsia, a serious condition affecting both mother and fetus.Maternal mortality and morbidity: Hypertension can lead to serious health problems for the mother, including stroke, heart attack, and kidney failure.Fetal growth restriction: If the mother's blood pressure is high, it can restrict blood flow to the baby, limiting its growth and development. Preterm birth: High blood pressure can also increase the risk of delivering the baby too early[55].

GAP ANALYSIS

There's a clear gap in research on vitamin D and B12 deficiency during the first trimester in the north Indian population. The proposed study could be very valuable in addressing this gap and improving pregnancy outcomes in the region.

Vitamin D and Vitamin B12 deficiency are inter-related to pregnancy in first trimester .There are several studies on the association of vitamin D and vitamin B12 deficiency in pregnant women in first trimester in south Indian population and Asian population and few studies on how vitamin D and vitamin B12 deficiency exposure involved in fetus and pregnant lady in first trimester. But there is no data regarding vitamin D and vitamin B12 deficiency in pregnancy in first trimester with mother and fetus effect on during reproductive age because none of the work is carried out among north Indian population. So this study will help us to find the association of vitamin D and vitamin B12 deficiency during pregnancy in first trimester is effect on mother and fetus on during reproductive age in north Indian population.

RESEARCH QUESTION

To explore the relationship vitamin D & vitamin B12 status during first trimester of pregnancy in tertiary care centre.

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HYPOTHESIS

There is potential role of vitamin D and B12 during the first trimester of pregnancy,

Vitamin D: The proper development and growth of fetal bones may be dependent on vitamin D sufficiency. Studies suggest a link between maternal vitamin D deficiency and the risk of small-for-gestational-age (SGA) births.

Vitamin B12: Vitamin B12 plays a critical role in pregnancy, supporting the development of the fetus and the mother's overall health

The general hypothesis is that adequate levels of vitamin D and B12 during the first trimester might contribute to a healthier pregnancy by:Supporting fetal development, Promoting placental function, Reducing the risk of certain pregnancy complications

NULL HYPOTHESIS

There is no significant link between Vitamin D and Vitamin B12 in first trimester of pregnancy. vitamin D and vitamin B12 aren't directly related in terms of their functions.

AIM AND OBJECTIVES

The aim of the current study is to analyze Vitamin D and vitamin B12 status in pregnant women in first trimester and to identify to adverse outcomes in mother and fetus in base of their deficiency.

OBJECTIVE:-

1. To examine the impact of vitamin D levels during the initial phase of pregnancy.

2. To examine the impact of vitamin B12 levels in the initial phase of pregnancy.

3. To analysis the association of vitamin D and vitamin B12 level in first trimester of pregnancy.

MATERIALS AND METHODS

PRINCIPLE: ECLIA Cobas e 411 VITAMIN D & VITAMIN B12 KIT

For Vitamin B12 Estimation

The estimation of Vitamin B12 levels in human serum or plasma was carried out using the Elecsys Vitamin B12 II assay based on the electrochemiluminescence immunoassay (ECLIA) method. The principle of the assay follows a competitive binding process where Vitamin B12 from the sample competes with biotinylated Vitamin B12 for binding sites on the ruthenium-labeled intrinsic factor, a Vitamin B12-binding protein. The assay steps involved are as follows:

Test Principle

The Elecsys Vitamin B12 II assay follows a competition principle. The procedure involves multiple incubation steps and utilizes chemiluminescence for detection. The assay's total duration is 27 minutes.

1. **First Incubation**: 15 µL of the sample is mixed with two pretreatment reagents (PT1 and PT2) to release bound vitamin B12.
2. **Second Incubation**: The pretreated sample is incubated with ruthenium-labeled intrinsic factor, allowing vitamin B12-binding protein complexes to form. The complex concentration depends on the vitamin B12 level in the sample.
3. **Third Incubation**: Streptavidin-coated microparticles and biotin-labeled vitamin B12 are added. Unbound binding sites on the intrinsic factor become occupied, and a ruthenium-labeled intrinsic factor–vitamin B12–biotin complex forms. This complex binds to the solid phase via streptavidin-biotin interaction.

   The reaction mixture is aspirated into a measuring cell, where microparticles are magnetically captured on an electrode's surface. Unbound substances are removed, and chemiluminescent

emission is induced by applying voltage to the electrode. The emitted light is measured by a photomultiplier, and results are calculated using a calibration curve generated instrument-specifically by two-point calibration and a master curve.

Reagents

The reagent rackpack and pretreatment reagents labeled as B12 II contain the following components:

- **PT1** (Pretreatment reagent 1, white cap): Dithiothreitol 1.028 g/L, stabilizer, pH 5.5.
- **PT2** (Pretreatment reagent 2, gray cap): Sodium hydroxide 40 g/L, sodium cyanide 2.205 g/L.
- **M** (Streptavidin-coated microparticles, transparent cap): Streptavidin-coated microparticles 0.72 mg/mL, preservative.
- **R1** (Intrinsic factor~Ru(bpy), gray cap): Ruthenium-labeled recombinant porcine intrinsic factor 4 µg/L, cobinamidedicyanide 15 µg/L, stabilizer, human serum albumin, phosphate buffer, pH 5.5, preservative.
- **R2** (Vitamin B12~biotin, black cap): Biotinylated vitamin B12 25 µg/L, biotin 3 µg/L, phosphate buffer, pH 7.0, preservative.

Equipment and Accessories Required (Not Provided)

- **Vitamin B12 II CalSet** for calibration
- **PreciControlVaria** for quality control
- **General laboratory equipment**
- **MODULAR ANALYTICS E170** or **cobas e analyzer** and necessary accessories, such as system buffers, washwater additives, reaction cups, and pipette tips

Specimen Collection and Preparation

- Acceptable specimens: Serum or plasma (Na-heparin, Li-heparin, K2-EDTA, K3-EDTA).
- Specimen stability:
  - 2 hours at 20-25 °C

- 48 hours at 2-8 °C
- 8 weeks at −20 °C
- Serum in separating tubes: 24 hours at 2-8 °C
- Avoid hemolyzed samples due to falsely low results.
- Samples from patients on high biotin therapy (> 5 mg/day) should be collected at least 8 hours post-administration.

Calibration and Quality Control

- **Calibration**: Based on a two-point calibration using the **Vitamin B12 II CalSet** and a master curve.
- **Quality Control**: PreciControlVaria or equivalent control material should be run at least once every 24 hours or following each calibration.

Calculation and Conversion Factors

- The analyzer calculates vitamin B12 concentration in pg/mL or pmol/L.
- Conversion factors:
  - pmol/L × 1.36 = pg/mL
  - pg/mL × 0.738 = pmol/L

Measurement Range

- **Measuring Range**: 150-2000 pg/mL or 111-1476 pmol/L.
- Values below the limit of quantitation are reported as <150 pg/mL, while values above the measuring range are reported as >2000 pg/mL.

Lower Limits of Measurement

- **Limit of Blank**: 50 pg/mL (36.9 pmol/L)
- **Limit of Detection**: 100 pg/mL (73.8 pmol/L)
- **Limit of Quantitation**: 150 pg/mL (111 pmol/L) with imprecision ≤ 20%.

For Vitamin D Estimation

The Elecsys Vitamin D total III assay is an immunoassay designed to quantitatively measure total 25-hydroxyvitamin D in human serum and plasma. This assay is intended to aid in assessing vitamin D sufficiency, particularly in adults, and is performed on cobas e immunoassay analyzers.

Test Principle:

1. **First Incubation**: The sample is treated with pretreatment reagents to release bound 25-hydroxyvitamin D from the vitamin D binding protein (VDBP).
2. **Second Incubation**: The sample is incubated with ruthenium-labeled VDBP. A complex forms between the 25-hydroxyvitamin D and the labeled VDBP. Cross-reactivity to 24,25-dihydroxyvitamin D is blocked by a specific antibody.
3. **Third Incubation**: Streptavidin-coated microparticles and biotinylated 25-hydroxyvitamin D are added. The complex binds to the solid phase via interaction of biotin and streptavidin. Chemiluminescent emission is measured to determine the concentration of 25-hydroxyvitamin D.

Summary:

- **Vitamin D** is a fat-soluble steroid hormone precursor, important for bone health. It is mainly produced in the skin and requires hydroxylation in the liver and kidney to become biologically active.
- **25-Hydroxyvitamin D** is the major storage form of vitamin D and is commonly used to determine overall vitamin D status. It has a half-life of 2-3 weeks and is predominantly in the form of 25-hydroxyvitamin D3.
- The assay also measures 25-hydroxyvitamin D2, which is less effective and present in lower amounts.

Reagents:

- **PT1**: Pretreatment reagent 1.
- **PT2**: Pretreatment reagent 2.

- **M**: Streptavidin-coated microparticles.
- **R1**: Vitamin D binding protein labeled with ruthenium.
- **R2**: Biotinylated 25-hydroxyvitamin D.

Precautions:

- For in vitro diagnostic use only.
- Follow safety instructions for handling reagents, including wearing protective equipment and following disposal guidelines.
- Reagents should be stored at 2-8°C and not frozen.

Calibration and Quality Control:

- Calibration must be performed once per reagent lot.
- Use PreciControl Vitamin D total III for quality control.
- Controls should be run at least once every 24 hours when the test is in use.

Limitations and Interferences:

- The assay has been tested for interferences from endogenous substances and pharmaceutical compounds. No significant interference was observed up to the tested concentrations.
- High concentrations of biotin can affect the results.

Measuring Range:

- **6.00-120 ng/mL** (15.0-300 nmol/L). Values below 6.00 ng/mL are reported as < 6.00 ng/mL.

SAMPLE SIZE:-A total of 55 pregnant women (20-40 year) in first trimester of pregnancy were selected sample was collected for this study.

STUDY DESIGN:- It was a Cross sectional study on vitamin D & vitaminB12 status in 55 pregnant women (20-40 years) in first trimester.

PLACE OF STUDY:- This study was conducted in the department of biochemistry and department of obstetrics and gynecology of Santosh Medical College and Hospital,SDTU, Ghaziabad ,Uttar Pradesh to satisfy the inclusion criteria for the study

STUDY PERIOD:- 12 Months

INCLUSION CRITERIA

- Age between 20-40 years
- Vitamin D and Vitamin B12interconnected correlation in pregnant lady in first trimester.
- Patientswith informed written consent for enrollment was included in the study.

EXCLUSION CRITERIA

- Chronic liver disease/ Chronic kidney disease
- Acute liver disease/ Acute kidney disease
- Cardiovascular disease
- History of cancer
- Gestational Diabetes Mellitus
- Hepatitis B

STUDY PROTOCOL:

The study began with the collection of demographic and medical histories, including details on age, pregnancy status, and lifestyle factors such as dietary habits and sun exposure. Blood samples were analyzed for serum Vitamin D and B12 levels using the electrochemiluminescence assay (ECLIA) on the COBAS e411 analyzer as per the manufacturer's protocol. Data were coded and entered into a secure database for statistical analysis.

SAMPLE COLLECTION

The draw of blood was done by a healthcare professional. The use of these was intended to measure levels of vitamin D and B12. This type of study can provide valuable insights into the prevalence of vitamin D and B12 deficiency in a population and identify potential risk factors.

STATISTICALANALYSIS:

The study used a sample of 55 participants. Statistical analysis was conducted with a 95.6% confidence interval, p-value < 0.05, and 80% power. Parametric tests were applied to normally distributed data, and non-parametric tests for non-normal distributions. Categorical variables were analyzed using the Chi-square test. Correlations between Vitamin D and B12 levels were explored, with statistical significance set at $p < 0.05$.

Sample size formula: n=Z2P(1−P)d2n = \frac{Z^2 P(1-P)}{d^2}n=d2Z2P(1−P) Where:

Z = 1.96 (confidence level of 95.6%)

P = 0.05 (prevalence estimate)

d = 0.05 (absolute error)

Thus, n=55n = 55n=55.

$$\mathbf{n} = \frac{Z^2 P \sum}{d^2}$$

Where,

Z= Confidence level at 95.6% (standard value of 1.96)

P= Estimated Prevalence

$\sum$=(1-P)

D^2=Absolute Error , 5%

In our study, P=5%100=0.05

$\sum$=1-P = 1-0.05,= 0.95

Z=1.9

d= 0.05

$$n = \frac{1.9*0.05*0.9}{0.05^2}$$

n=55

SCOPE OF THE STUDY

The scope of this study focuses on examining the impact of vitamin D and vitamin B12 deficiencies on maternal and fetal health during pregnancy, as well as evaluating the effectiveness of supplementation. The study assesses the prevalence of these deficiencies, particularly in high-risk populations, and their associated complications, such as gestational diabetes, preeclampsia, anemia, and adverse fetal outcomes like preterm birth and low birth weight. Furthermore, it explores the role of supplementation in mitigating these risks by analyzing clinical trials and meta-analyses. Through this comprehensive review, the study aims to provide clinical recommendations for optimal vitamin supplementation during pregnancy and identify areas for further research to enhance maternal and neonatal health outcomes.

RESULTS

A total of 55 patients were included in the study, and they were according to age arranged to four different groups: the group are (n = 20-25, 26-30, 31-35, 36-40 patients). Data on demographics were shown in (Table 1).

Table 1: Frequency and Percentage of Age of pregnant lady in first trimester in north Indian population. N=55

Age	Frequency	Percent	Mean ± SD
20-25year	13	23.63%	30.93 ± 4.502 (Min. = 22, Max. = 40)
26-30year	28	50.90%	
31-35year	10	18.18%	
36-40year	4	7.27%	

Table 1&fig.1 show that average age of pregnant ladies was 30.93 years. The majority of 50.90% pregnant ladies had belonged to 26-30 years age group; 18.18% pregnant ladies belonged to 31-35 years age group; 7.27% pregnant ladies belonged to 36-40 years age group; and 23.63% pregnant ladies belonged to 20-25 years age group in this study.

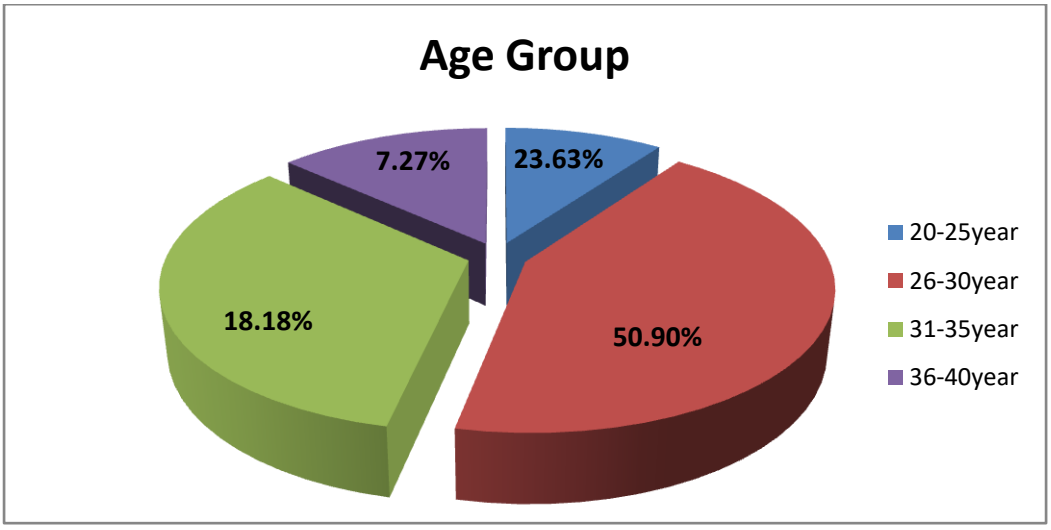

Fig.1

Table 2: Frequency and Percentage of vitamin D deficiency in pregnant lady in first trimester in north Indian population

N=55

Vitamin D (ng/ml)	Frequency	Percent	Mean ± SD
Deficient (<20ng/ml)	19	34.54 %	271.83 ± 159.362 (Min. = 10, Max. = 85)
Insufficient (21-29ng/ml)	21	38.18 %	
Sufficient (>30ng/ml)	15	27.27 %	

Table 2&fig.2 show that average vitamin D of pregnant ladies was 271.83ng/ml. The majority of 38.18% pregnant ladies had insufficient vitamin D; 34.54% pregnant ladies had deficient vitamin D; and 27.27% pregnant ladies had sufficient vitamin D in this study.

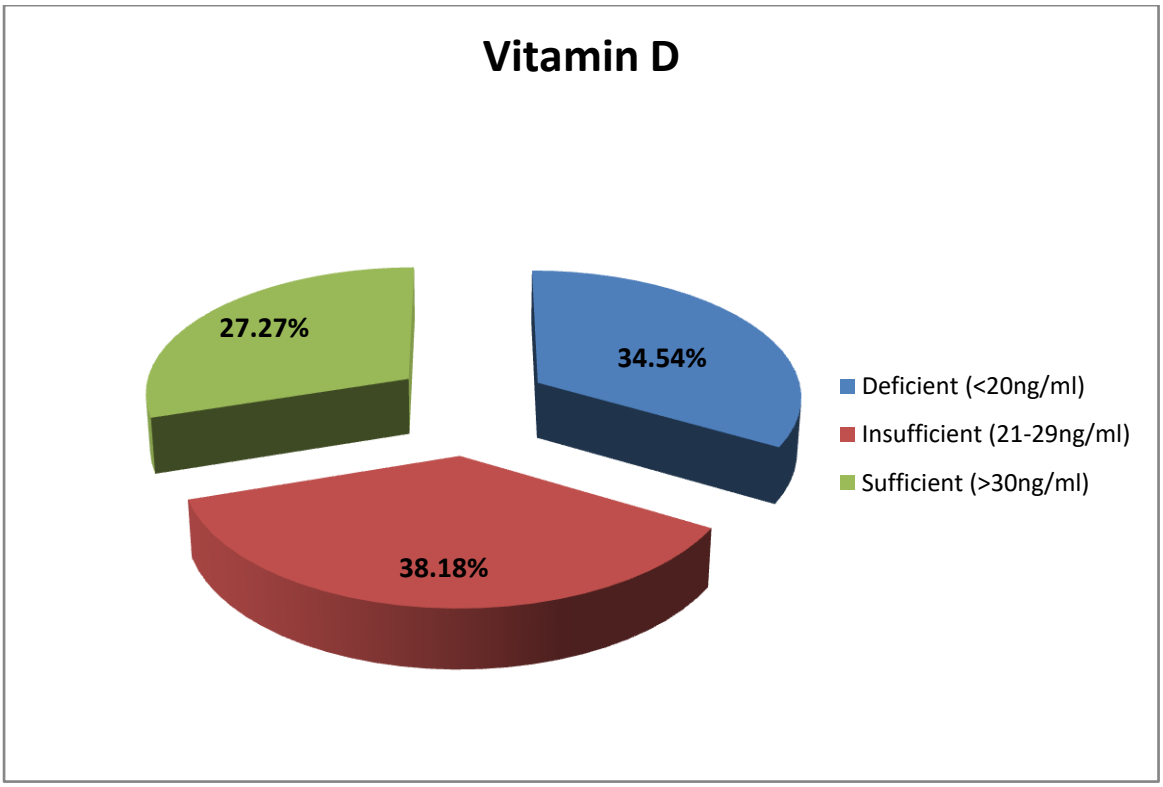

Fig.2

Table 3: Frequency and Percentage of vitamin B12 deficiency in pregnant lady in first trimester in north Indian population

N =55

Vitamin B12 (Pg/ml)	Frequency	Percent	Mean ± SD
Sufficient	51	92.72%	29.15 ± 18.145
High	4	7.27%	(Min. = 125, Max. = 951)

Table 3&fig.3 show that average vitamin B12 of pregnant ladies was 29.15pg/ml. The majority of 92.72% pregnant ladies had sufficient vitamin B12 and 7.27% pregnant ladies had high vitamin B12 in this study.

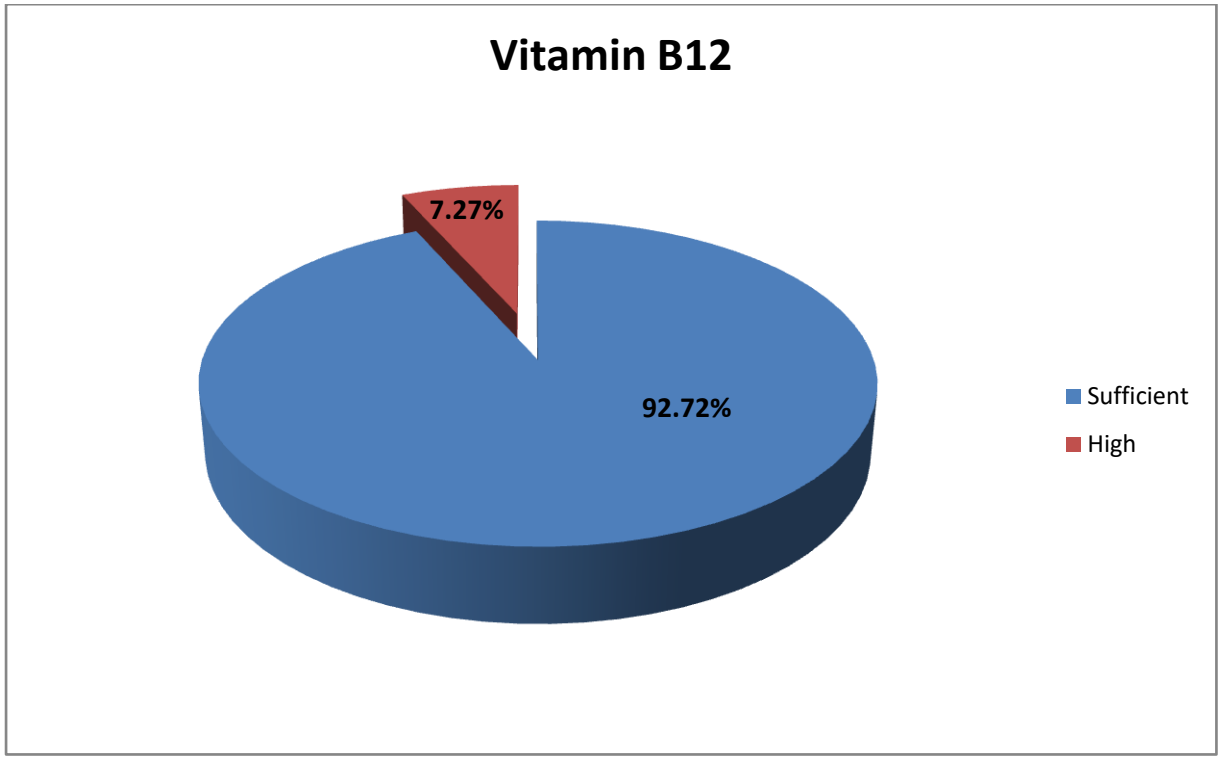

Fig.3

Table 4: Frequency and Percentage of Age wise vitamin D in pregnant lady in first trimester in north Indian population N=55

Vitamin D (ng/ml)	Age			
	20-25year (n=13) (%)	26-30year (n=28) (%)	31-35year (n=10) (%)	36-40year (n=4) (%)
Deficient	7 (53.85%)	8 (28.57%)	3 (30.0%)	2 (50.0%)
Insufficient	0 (0.0%)	14 (50%)	4 (40.0%)	0 (0.0%)
Sufficient	6 (46.15%)	6 (21.43%)	3 (30.0%)	2 (50.0%)

Table 4&fig.4 shows that 53.85% pregnant ladies had deficient vitamin D and 46.15% pregnant ladies had sufficient vitamin D in 20-25 years age group. 50% pregnant ladies had insufficient vitamin D and 21.43% pregnant ladies had sufficient & 28.57% pregnant ladies had deficient vitamin D in 26-30 years age group. 40% pregnant ladies had insufficient vitamin D and 30% pregnant ladies had sufficient & deficient vitamin D both in 31-35 years age group. 50% pregnant ladies had deficient & sufficient vitamin D both in 36-40 years age group in this study.

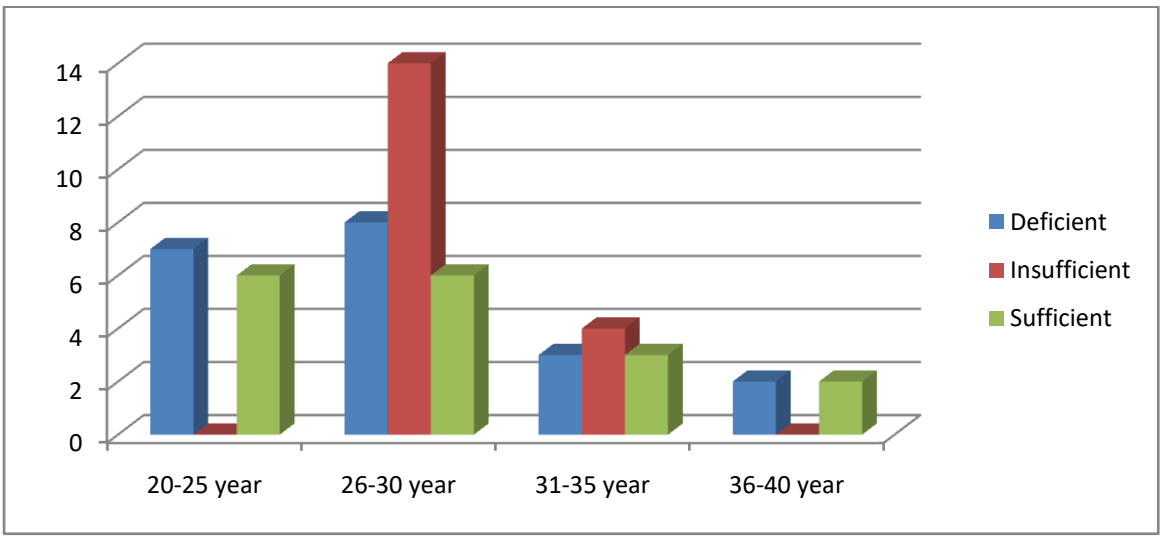

Fig.4

Table 5: Frequency and Percentage of Age wise vitamin B12 in pregnant lady in first trimester in north Indian population

N=55

Vitamin B12 (pg/ml)	Age			
	20-25year (n=13) (%)	26-30year (n=28) (%)	31-35year (n=10) (%)	36-40year (n=4) (%)
Sufficient	13 (100.0%)	28 (100.0%)	8 (75.0%)	1 (25.0%)
High	0 (0.0%)	0 (0.0%)	2 (25.0%)	3 (75.0%)

Table 5&fig.5 shows that 100% pregnant ladies had sufficient vitamin B12 in 20-25 years age group. 100% pregnant ladies had sufficient vitamin B12 in 26-30 years age group. 75% pregnant ladies had sufficient vitamin D and 25% pregnant ladies had high vitamin B12 in 31-35 years age group. 25% pregnant ladies had sufficient vitamin B12 and 75% pregnant ladies had high vitamin B12 in 36-40 years age group in this study.

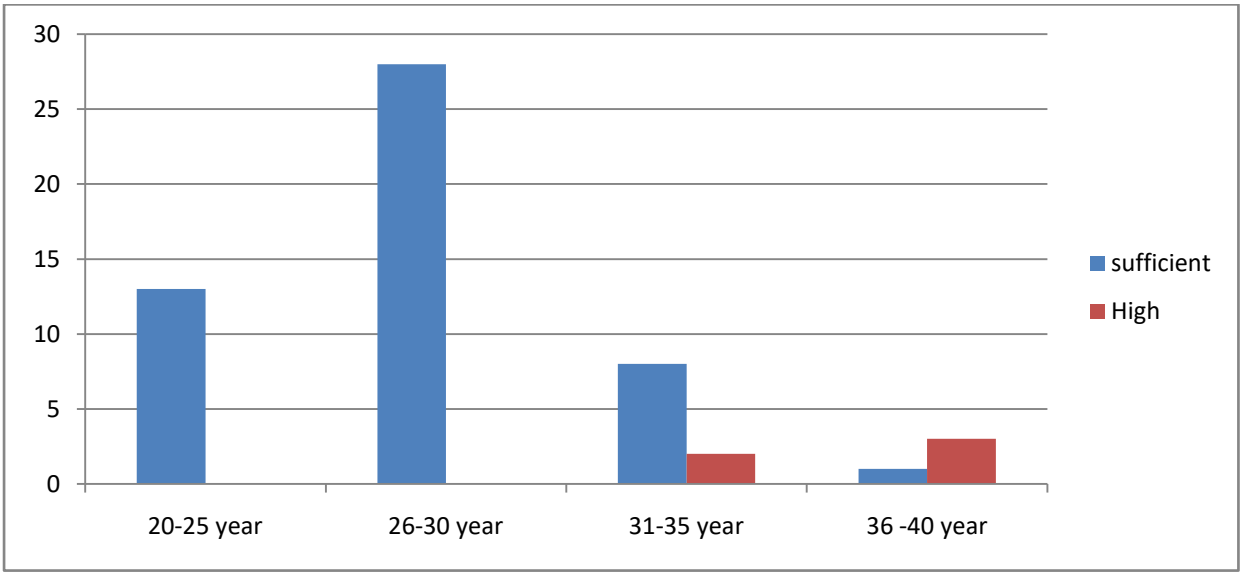

Fig.5

Table 6: Relationship between vitamins D and vitamin B12 deficiency in pregnant lady in first trimester in north Indian population

N=55

Vitamin B12	Vitamin D			Chi-square value	DF	P-value
	Deficient	Insufficient	Sufficient			
High	1 (5.27%)	1 (33.33%)	3 (8.82%)	1.25	2	0.535
Sufficient	18 (94.73%)	2 (66.66%)	31 (91.18%)			

Table 6&fig.6 shows that relationship between vitamins D and vitamin B12 deficiency in pregnant lady in first trimester in north Indian population. The majority of 94.73% pregnant ladies sufficient vitamin B12 and 5.27% pregnant ladies high vitamin B12 when they had deficient vitamin D. The majority of 66.66% pregnant ladies had sufficient vitamin B12 and 33.33% pregnant ladies high vitamin B12 when they had insufficient vitamin D. The majority of 91.18% pregnant ladies sufficient vitamin B12 and 8.82% pregnant ladies high vitamin B12 when they had sufficient vitamin D.

There was no relationship between vitamins D and vitamin B12 deficiency in pregnant lady in first trimester with P>0.05 in this study. , both are essential for overall maternal and fetal health.

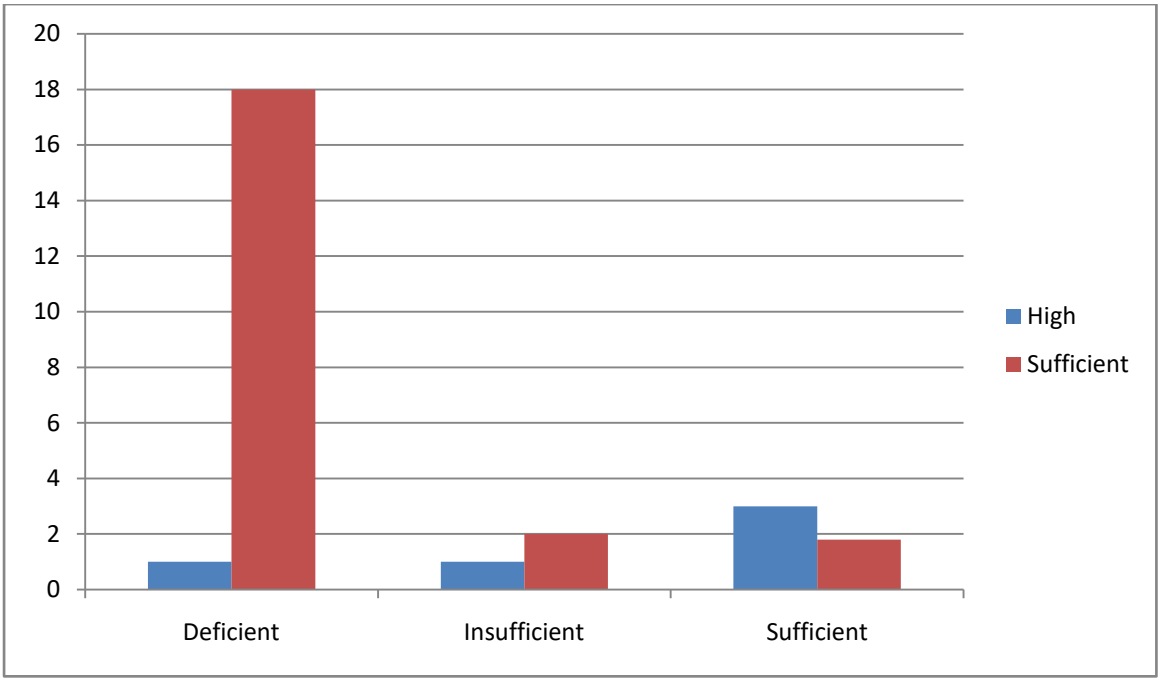

Fig. 6

There was no relationship between vitamins D and vitamin B12 deficiency in pregnant lady in first trimester with P>0.05 in this study.

Discussion

Our study highlights a significant prevalence of vitamin D deficiency in pregnant women during the first trimester, with 34.54% classified as deficient and 38.18% as insufficient. These findings are consistent with earlier research, particularly the study by **,**which reported a similar high prevalence of vitamin D deficiency in pregnant women from North India [56]**.** The persistence of this deficiency, despite abundant sunlight, can be attributed to lifestyle factors like limited outdoor activity and cultural practices [57]**.**observed that even in regions with high sunlight exposure, urbanized lifestyles and traditional clothing may impede adequate vitamin D synthesis^5. This underscores the need for targeted public health strategies to mitigate vitamin D deficiency, especially in pregnant women.

Vitamin D plays a crucial role in pregnancy, particularly in reducing the risk of pre-eclampsia, gestational diabetes, and preterm birth , emphasized that adequate vitamin D levels are essential for preventing complications like pre-eclampsia, gestational diabetes, and preterm labor[58]**.**Our findings echo those of who reported in a meta-analysis that vitamin D supplementation significantly reduces the risk of these adverse outcomes. Similarly, found a correlation between vitamin D deficiency and increased risk of pregnancy complications, further justifying the need for routine screening and supplementation [60]**.**This is further supported by, who demonstrated that vitamin D supplementation improves maternal and neonatal outcomes, reducing the risk of complications such as preterm birth and low birth weight [61]**.**

In line with our findings, also documented that vitamin D supplementation leads to improved pregnancy outcomes, including a lower incidence of complications [62]**.** These observations are consistent with the broader literature, such as the meta-analysis which highlighted that adequate vitamin D levels prevent adverse pregnancy outcomes [63]**.**Furthermoreshowed that vitamin D supplementation not only improves maternal bone health but also positively impacts neonatal outcomes[64]**.** Given the critical role of vitamin D, our study supports the growing consensus that screening and supplementation should be part of routine prenatal care to optimize pregnancy outcomes[65]**.**

On the other hand, our study observed a relatively lower prevalence of vitamin B12 deficiency, with only 7.27% of participants exhibiting low levels. This finding contrasts with previous studies, Who reported a higher prevalence of vitamin B12 deficiency, particularly in vegetarian populations in North India[66]**.**Have also highlighted that vegetarian diets significantly increase the risk of B12 deficiency, A factor that may account for the higher deficiency rates seen in some

cohorts. similarly identified vegetarianism as a major factor contributing to B12 deficiency in pregnant women [67].

Interestingly, our study did not find a significant correlation between vitamin D and B12 deficiencies, consistent with the findings of who also noted independent deficiency patterns for these vitamins [68],Emphasized the need for separate management strategies to address these deficiencies, given their distinct causes and implications for maternal and fetal health [69]. This is particularly important as vitamin D deficiency has been more strongly linked to adverse pregnancy outcomes, while vitamin B12 is primarily associated with preventing neural tube defects and maternal anemia [70].

While the role of vitamin D in pregnancy is well-documented, research on vitamin B12 supplementation's impact on broader pregnancy outcomes is less conclusive. found that B12 is vital for fetal neural development and the prevention of anemia, but its effect on other pregnancy outcomes is less pronounced compared to vitamin D. This was also observed that while B12 supplementation helps prevent anemia, it does not have the same wide-reaching impact on pregnancy outcomes as vitamin D [71]. However that further research is needed to fully understand the potential benefits of vitamin B12 supplementation during pregnancy [72].

In contrast to our findings of adequate B12 levels in most participants, higher deficiency rates in vegetarian populations, underscoring the need to consider dietary patterns when assessing B12 levels **[73]**. That in regions where animal-based diets are more common, B12 levels tend to be sufficient. This divergence suggests that population-specific dietary habits play a critical role in determining B12 status during pregnancy [74].

Future research should focus on longitudinal studies to assess how fluctuations in vitamin D and B12 levels throughout pregnancy impact maternal and fetal health. Emphasized the value of longitudinal studies in providing deeper insights into how these nutrient levels affect pregnancy outcomes [75]. Additionally, studies evaluating the effectiveness of different supplementation regimens and dietary interventions could help refine public health strategies, That supplementation strategies should be tailored to the specific needs of different populations, accounting for regional dietary practices and environmental factors [76].

In summary, our study reinforces the importance of monitoring vitamin D and B12 levels during pregnancy. While vitamin D deficiency poses a significant risk to various pregnancy outcomes, maintaining adequate vitamin B12 levels is equally crucial for optimal maternal and fetal health.

Routine screening, targeted supplementation, and public health interventions are essential to improving pregnancy outcomes and ensuring optimal maternal and fetal well-being [77].

Conclusion

This study underscores the significant prevalence of vitamin D deficiency among pregnant women in the first trimester, aligning with global and regional findings. Despite living in regions with adequate sunlight, lifestyle factors continue to impede sufficient vitamin D synthesis. The deficiency poses substantial risks to maternal and fetal health, including pre-eclampsia, gestational diabetes, and preterm birth, further justifying routine screening and supplementation during pregnancy. While the prevalence of vitamin B12 deficiency was comparatively lower in this cohort, maintaining adequate levels is essential for preventing maternal anemia and ensuring optimal fetal neural development. The absence of a strong correlation between vitamin D and B12 deficiencies highlights the need for individualized management strategies for each nutrient. This study advocates for public health initiatives that promote regular monitoring and supplementation of these vitamins during pregnancy, contributing to better maternal and neonatal outcomes. Future research should focus on the long-term impact of nutrient deficiencies throughout pregnancy and the effectiveness of tailored supplementation protocols.

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ANNEXURE-I

PROFORMA

(Details of patient to be included)

SANTOSH DEEMED TO BE UNIVERSITY (GHAZIABAD)

INFORMED CONSENT ENGISH

I_____ Son/ Daughter/ Wife of _____ resident_____,

give my full, free and voluntary informed consent in the thesis **"ANALYSIS OF VITAMIN D & VITAMIN B12 STATUS DURING 1st TRIMESTER OF PREGNANCY IN A TERTIARY CARE CENTRE "** Mr. Sahil raj has informed me to my full satisfaction in the language I understand, about the purpose, nature of the study and various laboratory investigations for the study to be carried out. I have been informed about the study duration and instructions to be followed during the study period.

I give full consent for being enrolled in the above study and I reserve my right to withdraw from the study, whenever I wish without my right to undergo for the treatment as Santosh Hospital, Ghaziabad, U.P.

Patient's/ Patient's Relative's Signature or

Doctor's Signature

Thumb impression

Name: Date:

We have witnessed that the patient signed the above in the presence of his free will after fully understanding the context.

Signature of witness

Name:

Signature of Investigator

Name: Sahil raj

M.Sc Medical Biochemistry

SMC&H, Ghaziabad.

ANNEXURE-II

SANTOSH DEEMED TO BE UNIVERSITY (GHAZIABAD)

INFORMED CONSENT HINDI

मैं _____बेटा/ बेटी/ पत्नी _____
निवासी_____ _____घोषणाकरताहूंकि साहिल राज नेमुझेमेरीभाषामेंअपनेअध्ययन"**ANALYSIS OF VITAMIN D & VITAMIN B12 STATUS DURING 1st TRIMESTER OF PREGNANCY IN A TERTIARY CARE CENTRE** ''केउद्देश्यकेबारेमेंऔरअध्ययनकेदौरानअपनाईजानेवालीविभिन्नप्रयोगशालाजांचकेबारेमेंसूचितकरदियाहै।मुझेअध्ययनकीअवधिऔरउसकेदौरानअपनाएजानेवालेनिर्देशभीमेरीभाषामेंसमझादिएहैं।मैंउपरोक्तअध्ययनमेंबिनाकिसीदबावकेदाखिलालेरहाहूंऔरपूर्णसहमतिदेरहाहूं।संतोषअस्पतालगाज़ियाबादमेंआगेउपचारपरबिनाप्रतिकूलप्रभावडालेंइसअध्ययनसेऔरप्रयोगशालाजांचकीप्रकृतिसेमैंजबचाहूंबाहरजानेकेअपनेअधिकारकोसुरक्षितरखरहाहूं।

रोगी / रोगीकेरिश्तेदारके हस्ताक्षरडॉक्टरकेहस्ताक्षर

नामदिनांक:

मेरेउपस्थितिमेंमरीजनेपूरीतरहडॉक्टरकेबताएगएअध्ययनऔरनिर्देशोंकोसमझनेकेबादअपनी स्वतंत्रइच्छासेऊपरकेफार्मपरहस्ताक्षरकिएहैं।

हस्ताक्षर:

नाम:साहिलराज
पदनाम: एमएससीमेडिकलबायोकेमिस्ट्री

संतोषमेडिकलकॉलेजएवंअस्पतालगाजियाबाद

ANNEXURE-III

SANTOSH DEEMED TO BE UNIVERSITY

Santosh Nagar, Pratap Vihar, Ghaziabad, UP 201009

(Department of Biochemistry)

CASE REPORT FORM	
DEMOGRAPHIC DATA	
Name	Mr./Mrs./Ms.:-
Date of Birth:-	Sex: Male/Female
Weight (in Kg):-	Height (in cm):-
Address:	
Street:_____City:_____ State:_____ Pin Code:_____	
Mobile No.	: Email:
Education Status:	
Occupation:	

LETTER OF ETHICAL CLEARANCE FOR DISSERTATION FROM REGISTRAR OFFICE

SANTOSH
Deemed to be University
(Established u/s 3 of the UGC Act, 1956)

F. No. SU/R/2024/1344[10] Dated: 17.05.2024

Subject: Grant of Ethical Clearance for **Dissertation Protocol** Topic **"Vit- D and Vit- B 12 Status During 1st Tremester of Pregnancy",** submitted by **SAHIL RAJ,** student of M.Sc. Medical BIOCHEMISTRY, 2021 Batch.

*****

With reference to the request for grant of Ethical Clearance for the Dissertation Protocol entitled **"Vit- D and Vit- B 12 Status During 1st Tremester of Pregnancy", SAHIL RAJ** is informed that the **Dissertation Protocol** submitted by him/her was considered by the **Screening Committee** of the **Allied Health Sciences** in its meetings held on 07.02.2024 and 29.02.2024. The recommendations of the **Screening Committee** were considered in detail by the **Institutional Ethics Committee** in its meeting held on **15.05.2024** and the same was **approved** by the **Ethics Committee** of the **Santosh Deemed to be University, Ghaziabad, Delhi NCR.**

He /She is informed accordingly for further necessary action.

17/5/24.

[Dr. EZHILRAJAN. K]
REGISTRAR

REGISTRAR

SAHIL RAJ
M.Sc. Medical BIOCHEMISTRY.

Copy to:

1. The Vice Chancellor
2. The Dean – Academics
3. The Dean, SMC&H
4. The Dean – Research
5. The Director - IQAC
6. HOD/ Course In-charge of the concerned Department.

STATISTICIAN CERTIFICATE

This is to certify that the statistical analysis component of the thesis titled **"ANALYSIS OF VITAMIN D & VITAMIN B12 STATUS DURING 1st TRIMESTER OF PREGNANCY IN A TERTIARY CARE CENTRE ''** done by **Mr. Sahil raj,** in the Department of Biochemist, Santosh Medical College & Hospital, Ghaziabad, has been conducted under my guidance and supervision.

All statistical calculations of this study are authentic and correct to the best of my knowledge.

I wish his all the success in life.

Signature:

Date:

Place: Santosh Medical college
Ghaziabad: 201009

Vikash Singh Patel, MSc

Assistant Professor cum Statistician,

Department of Community Medicine,

Santosh Medical College,

Ghaziabad: 201009

ANALYSIS OF VITAMIN D & VITAMIN B12 STATUS DURING 1st TRIMESTER OF PREGNANCY IN A TERTIARY CARE CENTRE

ORIGINALITY REPORT

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MASTER CHART

S.NO.	AGE(year)	WEIGHT (Kg)	VITAMIND (ng/ml)	VITAMIN B12 (pg/ml)
1.	22	38	15	288
2.	29	43	13	150
3.	26	37	29	215
4.	26	39	39	198
5.	38	55	38.3	139
6.	28	41	60	699
7.	28	47	28	209
8.	35	41	34.6	366
9.	32	54	85	506
10.	27	39	47	214
11.	29	43	38	185
12.	30	58	29	333
13.	40	53	26	499
14.	25	49	13	951
15.	29	65	16	220
16.	35	59	23	228
17.	35	48	29	119
18.	27	58	77	193
19.	39	65	28	441
20.	36	59	13.77	209
21.	29	47	12	228
22.	39	58	10	199
23.	25	39	33	193
24.	34	58	21	411
25.	39	61	25	209
26.	29	54	16	206
27.	33	57	25	205
28.	31	61	28	223
29.	35	48	27	196
30.	22	39	18	205

S.NO.	AGE(year)	WEIGHT (Kg)	VITAMIND (ng/ml)	VITAMIN B12 (pg/ml)
31.	23	38	16	125
32.	21	58	15	206
33.	24	50	17	186
34.	23	51	18	289
35.	21	37	15	368
36.	35	59	23	228
37.	29	62	23	265
38.	22	53	17	209
39.	24	60	18	199
40.	23	57	17	438
41.	27	54	28	217
42.	29	47	27	298
43.	26	39	36	368
44.	28	63	27	478
45.	29	54	26	256
46.	28	61	29	216
47.	26	38	32	408
48.	27	67	29	398
49.	27	54	34	208
50.	29	38	37	198
51.	30	43	30	398
52.	30	53	28	438
53	29	64	38	277
54	27	46	26	388
55	30	59	19	202